Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(8):137-142

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis Characterisation and Antibacterial Activity of Photoresponsive Silver Nanomaterial against *E. coli*

M Giridhar[1], HS Bhojya Naik[1*], CN Sudhamani[1], MC Prabhakar[1], R Kenchappa[1] and Sameer Patil[2]

[1]*Department of PG Studies and Research in Industrial Chemistry, School of Chemical Sciences, Kuvempu University, Karnataka, India*
[2]*Department of PG Studies and Research in Biochemistry, Kuvempu University, Karnataka, India*

ABSTRACT

In this paper, we report the synthesis and antibacterial activity of 4-[(E)-(5-ethyl-1,3,4-thiadiazol-2-yl)diazenyl]phenol-alkanethiol functionalized silver nanoparticle(Ag@Azodye). The synthesised nanoparticles were characterized by UV–Vis absorption spectroscopy, XRD spectra, SEM and TEM analysis. Results showed that the functionalized silver Nanoparticles to be spherical having size 9.5-16 nm, from UV-Vis absorption studies it can be observed that, the azodye undergoes photoisomerisation on the surface of silver nanoparticle upon irradiation of 365 nm UV light. The antibacterial activity of functionalized silver nanoparticles against E. coli was investigated in a nutrient broth supplemented with different concentrations of functionalized silver particles under dark and photo irradiated condition. These particles were found to be an effective bactericide and their activity increased upon irradiation of UVA light.

Keywords*:* Azo dye; Photoresponsive silver nanoparticle; Photoinduced antibacterial activity

---

INTRODUCTION

Azo dyes are important class of organic dyes, which are finding applications in many fields [1-4], over past few decades the antimicrobial properties of heterocyclic azo dyes have been explored. Textile materials undergo degradation due to the activity of fungi and bacteria which results in the loss of mechanical strength, colour etc, and antimicrobial azodyes could prevent the damage of cloth and increases the shelf life of cloth [5,6]. The photoresponsive property of azodye is much fascinating; when stable trans isomer of azobenzene is exposed to UV light (365 nm) it isomerizes to the cis form. The cis isomer is metastable; it spontaneously reverts back to the trans form once the UV source is removed [7]. The introduction of Azodye photo-switched organic molecule onto inorganic nanoparticle surface is good strategy for developing light-controlled nano-devices. Azobenzene sensitized silver nanoparticle are finding application in optical [8,9] and magnetic data storage device [10]. To our knowledge azodye sensitized silver nanoparticle has never been used for exploration of antibacterial activity. Silver nanoparticle has been known to be an effective antibacterial agent against a broad range of microorganisms, the silver ions released from metallic silver or from the surfaces of nanoparticles can interact with thiol groups in bacterial proteins or by interfering with DNA, silver ions can also interact with the cell wall causing cell lysis [11,12]. In recent years several functionalized silver nanoparticles have been developed which showed improved antibacterial activity relative to silver nanoparticles, some of the functionalized silver nanoparticle which were tested for antibacterial activity include norvancomycin capped silver nanoparticles [13], oleic acid functionalized silver nanomaterial [14], ampicilin functionalized silver nanomaterial [15], glycoprotein capped silver nanomaterial [16], though the functionalization of silver nanoparticle improves the antibacterial activity of silver nanoparticle its mechanism is not known.

In the present work we have synthesised heterocyclic azodye with alkanethiol anchoring group which undergoes photoisomerisation upon irradiation of 365 nm UV light, functionalized silver nanoparticle were studied for their photoinduced antibacterial activity. The heterocyclic moiety in azodye being 1,2,4- thiadiazole, literature

reviews has proved that the thiadiazole scaffold possess broad spectrum of pharmacological activities like antimicrobial, antifungal, anti-inflammatory, anticancer, anticonvulsant, antidepressant and antioxidant activities[17,18].

EXPERIMENTAL SECTION

Materials

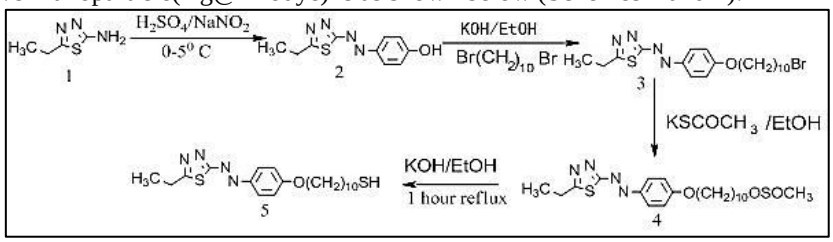
Silver nitrate, potassium hydroxide, hexane, diethyl ether and Phenol were obtained from (SD Fine-Chem Pvt. Ltd, India). Ethanol, 1, 10-dibromo decane, tetraoctylammonium bromide, thioacetic acid are purchased from Aldrich Chemicals. Potassium thioacetate was prepared in laboratorory using standard procedure from thioacetic acid and potassium hydroxide.

Spectroscopic Measurements

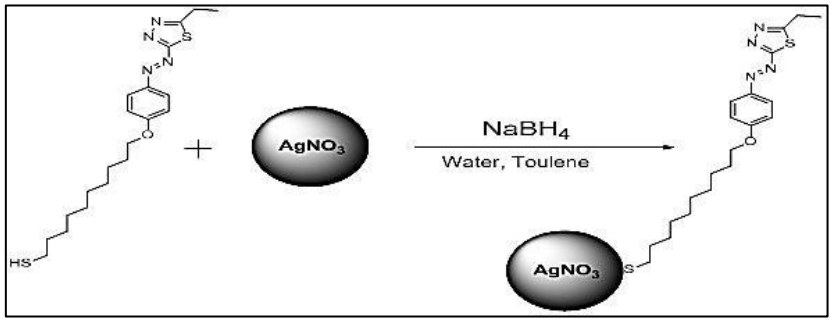
^{1}H NMR spectra were measured on a 400 MHz Bruker Avance 400 NMR spectrometer in DMSO-d_6. Mass spectra were obtained on Micromass (UK) Platform II GC/LC-Mass Spectrometer. IR spectra were measured on Midac Prospect FT-IR spectrometer. The evaluation of structure and crystal phase was done by powder X-ray diffraction (XRD), which was measured on Shimadzu X-ray diffractometre equipped with CuKα (λ=1.5406Å) radiation operating in the 20 range of 20°-80° with a scan rate of 1° min^{-1}. Scherer equation was applied for the major XRD peak, TEM image was obtained on JEM-2010 (JEOL, 200 kV) TEM. Absorption spectra were recorded on a Shimadzu UV-2401PC spectrophotometer. Reaction progress of photoisomerisation on UV irradiation and reverse thermal isomerisation in the dark was monitored by change of absorption spectra.
The antibacterial activity of the given compound was tested on the Gram negative bacteria *Escherichi coli* (ATCC 25922). The antibacterial media was prepared from 0.25 gm beef extract, 0.30 gm peptone and 100 ml water. Nanoparticles in one set were weighed 0 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml and 64 µg/ml. They were dissolved in 50 ml conical flask containing 25 ml of the media. It was kept in rotating shaker at 120 rpm at 25°C temperature. Reading for each of them were taken on 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12 hrs.
In another set the nanoparticles dissolved in media was irradiated with 365 nm wavelength light. Activity was compared both for irradiated and non-irradiated samples by measuring the optical density at 600 nm using shimadzu spectrophotometer.

Synthesis

The synthetic route for the synthesis of 4-[(E)-(5-ethyl-1,3,4-thiadiazol-2-yl)diazenyl]phenol-alkanethiol functionalized silver nanoparticle(Ag@Azodye) is as shown below (Schemes 1 and 2).



Scheme 1: Synthetic route for the synthesis of 4-[(E)-(5-ethyl-1,3,4-thiadiazol-2-yl)diazenyl]phenol-alkanethiol



Scheme 2: synthetic route for the synthesis of 4-[(E)-(5-ethyl-1,3,4-thiadiazol-2-yl)diazenyl]phenol-alkanethiol functionalized silver nanoparticle

Synthesis of (E)-2-((4-((10-bromododecyl)phenyl)diazenyl)-5-ethyl-1,3,4-thiadiazole (3):

To a solution of 4-[(E)-(5-ethyl-1,3,4-thiadiazol-2-yl)diazenyl]phenol (2.34 g, 0.01 mol) in deoxygenated ethanol (50 mL) was added to a solution of potassium hydroxide (20 mL, 0.5 M, 0.01 mol) in deoxygenated ethanol. After refluxing the mixture for 30 min, a solution of 1,10-dibromodecane (3.0 g, 0.01 mol) in deoxygenated ethanol (40 mL) was added dropwise for 30 min to the reaction mixture and the resulting solution

was refluxed for an additional 5 hours. As the solution cooled, it became cloudy with a KBr precipitate. The reaction mixture was concentrated. The crude mixture was purified with silica gel column chromatography eluted with a 1:9 ether/hexane. An orange crystalline solid was isolated (1.58 g, yield 35%).
IR: 1550 cm^{-1}, 3080 cm^{-1}, 2950 cm^{-1}, 1230 cm^{-1}, ^{1}H NMR: 1.4(8H, m, -$OCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2$-), 1.5(7H, m, -$CH_3$, -$OCH_2CH_2CH2$-, -$CH_2CH_2CH_2Br$), 1.8(4H, m, -$OCH_2CH_2$-, -$CH_2CH_2Br$) , 3.1(2H, q, -$CH_2CH_3$), 3.4(2H, t, -$CH_2Br$), 4.1 (2H, t, –$OCH_2CH_2$-), δ 7.0 (2H, d), 8.0 (2H, d), MS *m/z* 455 [*M+1*]. Found, %: C 52.98, H (6.45%) Br 17.62, N 12.36, O 3.53, S 7.07, Calculated, %: C 52.94, H(6.45%) Br 17.66, N 12.30, O 3.59, S 7.07.

Synthesis of (E)-S-(10-(4-((5-ethyl-1,3,4-thiadiazol-2-yl)diazenyl)phenoxy)decyl)ethanethiolate (4):
To a solution of compound 3 (1.5 g, 3.3 mmol) in deoxygenated ethanol (50 mL) was added potassium thioacetate (0.36 g, 3.6 mmol). After refluxing the mixture for 24 hr, the solvent was evaporated. The crude mixture was purified with silica gel column chromatography eluted with a 1:9 ether/hexane. An orange crystalline solid was isolated (0.67 g, yield 41%). IR: 2575 cm^{-1}, 1658 cm^{-1}, 3080 cm^{-1}, 1553 cm^{-1}, 2980 cm^{-1}, 1070 cm^{-1}, 970 cm^{-1}, ^{1}H NMR: δ 7.0 (2H, d), 8.0 (2H, d), 4.1 (2H, t, –OCH_2CH_2-), 3.2 (2H, t, -CH_2Br), 2.8 (2H, q, -CH_2CH_3), 1.8 (2H, s, -$SCOCH_3$), 1.5-1.3 (21H, m, -CH_2CH_3, O-CH2$CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2$-), MS *m/z* 450 [*M+1*]. Found, %: C 55.72, H 7.13, N 12.38, O 10.60, S 14.17, Calculated, %: C 55.62, H 7.23, N 12.40, O 10.68, S 14.27.

Synthesis of (E)-10-((4-((5-ethyl-1,3,4-thiadiazol-2-yl)diazenyl) phenoxy)decane-1-thiol (5):
To a solution of compound 4 (0.6 g, 1.3 mmol) in deoxygented ethanol (30 mL) was added a solution of potassium hydroxide (3.2 mL, 0.5 M, 1.8 mmol) in deoxygenated ethanol. After stirring the mixture for 1 hr, a solution of ammonium chloride (6.8 mL, 1.0 M, 6.8 mmol) in deoxygenated water was added. The resulting solution was concentrated and extracted with diethyl ether. The ether solution was washed first with water and then with saturated aqueous sodium chloride solution, dried over magnesium sulfate and filtered. The solvent was evaporated. The crude mixture was purified with silica gel column chromatography eluted with a 1:9 ether/hexane. An orange solid was isolated (0.22 g, yield 41%).
IR: 2570 cm^{-1}, 1658 cm^{-1}, 3080 cm^{-1}, 1550 cm^{-1}, 2980 cm^{-1}, 1068 cm^{-1}, 970 cm^{-1}, ^{1}H NMR: δ 7.0 (2H, d), 8.0 (2H, d), 4.1 (2H, t, –OCH_2CH_2-), 2.5 (2H, t, -CH_2SH), 3.1 (2H, q, -CH_2CH_3), 1.4 (8H, m, -$OCH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2$-), 1.5 (7H, m, -$CH_3$, -$OCH_2CH_2CH2$-, -$CH_2CH_2CH_2Br$), 1.8 (4H, m, -$OCH_2CH_2$-, -$CH_2CH_2Br$), 1.6 (1H, m,-SH). MS m/z 408 [M+1]. Found, %: C 59.08, H 7.44, N 13.78, O 3.93, S 15.77, Calculated, %: C 59.18, H 7.34, N 13.80, O 3.95, S 15.73.

Preparation of (E)-10-((4-((5-ethyl-1,3,4-thiadiazol-2-yl)diazenyl) phenoxy)decane-1-thiol -capped silver nanoparticle (6):
A solution of $AgNO_3$ (0.27 g, 1.6 mmol) in water (40 mL) was mixed with a solution of tetraoctylammonium bromide (1.44 g, 2.6 mmol) in toluene (140 mL). The two-phase mixture was vigorously stirred until all the silver ions was transferred into the organic layer and compound 5(649 mg, 1.6 mmol) was added to the organic layer. A freshly prepared solution of sodium borohydride (2.8 g, 75 mmol) in water (140 mL) was slowly added with vigorous stirring. After further stirring for 3 hrs, the organic layer was separated and the solution was filtered and washed with acetonitrile. A dark brown solid was obtained (0.16 g).
IR:1658 cm^{-1}, 3080 cm^{-1}, 1550 cm^{-1}, 2980 cm^{-1}, 1068 cm^{-1}, 970 cm^{-1}.

RESULTS AND DISCUSSION

UV-Vis Absorption Studies

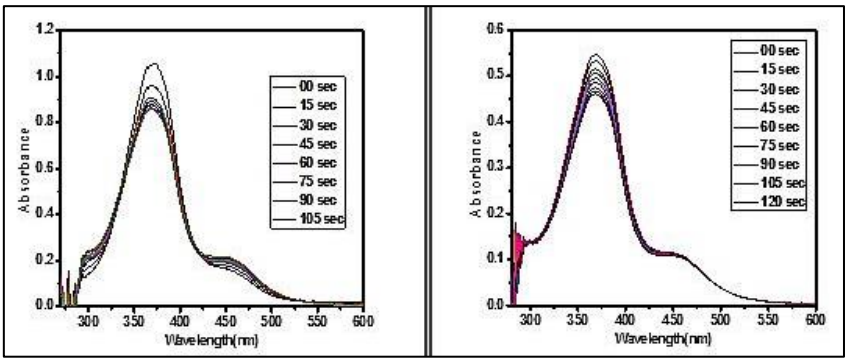


Figure 1: Shows the changes in the UV-Vis spectrum resulting from the isomerisation of azodye sensitized silver nanoparticles

Photoisomerisation of compound 5 on the surface of silver nanoparticle was studied by irradiating a sample of a nanoparticle in aqueous solution with light of 365 nm wavelength as shown in the Figure 1. The wavelength of

maximum absorption of nanoparticle was same as organic compound. The UV-spectra of the functionalized silver nanoparticle showed two characteristic peaks at 370 and 459 nm, which is at higher wavelength when compare to azobenzene with characteristic peaks at 338 and 438 nm [17]. In general for most of biological applications the photoswitching property of azobenzene should be in visible region. When stable trans isomer of functionalized nanoparticle in dichloromethane when exposed to UV (λ=365 nm) light, it isomerised to the cis form (Figure 1). The UV spectrum of trans compound 5 has a strong π-π^* band with a λ_{max} of 370 nm and a weaker n-π* band near 459 nm measured in dichloromethane.

Morphological Characterisation by TEM

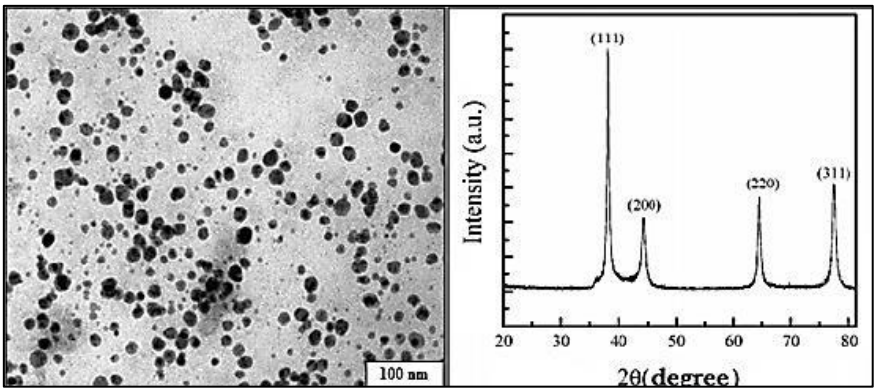


Figure 2: Shows TEM images of silver nanoparticles functionalised with azodye and XRD images of silver nanoparticles functionalised with azodye

Transmission electron microscopic (TEM) images in Figure 2 of Thiadiazole-azobenzene-alkanethiol functionalized silver nanoparticle showed, these images indicate nearly spherical shape and very narrow size distribution, particle size in diameter range of 9.5-16 nm. The analysis of the crystal structure and phase was done by powder X-ray diffraction. Figure 2 shows the XRD pattern for the azodye functionalized silver nanoparticles. The XRD spectra shows broad peaks at the positions at 38.3°, 44.6°, 64.8°, 77.6° and 81.9°corresponding to the (111), (200), (220) and (311) structure of metallic silver and these peak position are in good agreement with the standard files of JCPDS (PDF card 04-0783) suggesting that these samples of crystal are fcc in nature. Absences of other diffraction peaks other than silver indicate high purity of the synthesized products. The estimated crystallite size of azodye sensitized silver nanocrystals were 15.8 nm according to Scherer equation.

$$D=K\lambda / \beta \cos \theta \text{ ---- (1)}$$

Where, K is a constant equal to 0.89, λ is the X-ray wavelength and β is the full width at half maxima.

Antibacterial Activity

Bacteriological tests were performed in LB medium in liquid systems using broth and agar dilution method supplement with the control sample. The dynamics of bacterial growth was monitored in liquid LB medium by measuring the optical density at 600 nm using shimadzu spectrophotometer. This result confirmed that azodye functionalized silver nanoparticle owns enhanced antibacterial activity against Gram-negative *E. coli*. We can observe a significant difference in activity with non-irradiated and irradiated forms from the graph (Figure 3) it can be observed that non irradiated functionalized silver nanoparticle does not show any significant activity for 0 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml and 64 µg/ml, it could be concluded that MIC for non-irradiated is much higher than 32 µg/ml. The corresponding MIC of functionalized silver nanoparticles under photo irradiation where 100% reduction of microbial growth is seen for 32 µg/mL and hence this is considered to be MIC for functionalized Ag NPs under photo irradiation nutrient broth solution. However, future studies on the biocidal influence of this nanomaterial on other Gram positive and Gram-negative bacteria are necessary in order to fully evaluate its possible use as a new bactericidal material.

In order to understand the mechanism of antibacterial action of azodye sensitized silver nanoparticle, the release of Ag^+ ions in solution was studied by irradiating a solution of nanoparticle in 50 ml of pure distilled water with UVA lamps having spectral bands cantered at 350 nm over a period of 2 days, fused quartz 50 mL Erlenmeyer flasks were used to achieve maximum UV light transmittance. Flasks containing nanoparticle concentration of 16 mg/L and 32 mg/L were prepared in duplicate. One of the flasks was placed on a rotary platform shaker operated at 80 rpm to ensure uniform mixing with light exposure. The other flask used as dark control placed under identical conditions wrapped in aluminium foil. An aliquot of 1 ml sample was removed at the interval of 8 hr, centrifuged at 5000 rpm the clear solution was analysed for dissolved Ag^+ ions using Graphite furnace-

atomic absorption spectroscopy (GF-AAS, Thermo Scientific) having detection limits of 0.8 µg L^{-1}. The results are shown below for concentration 16 mg/L and 32 mg/L of nanoparticles.

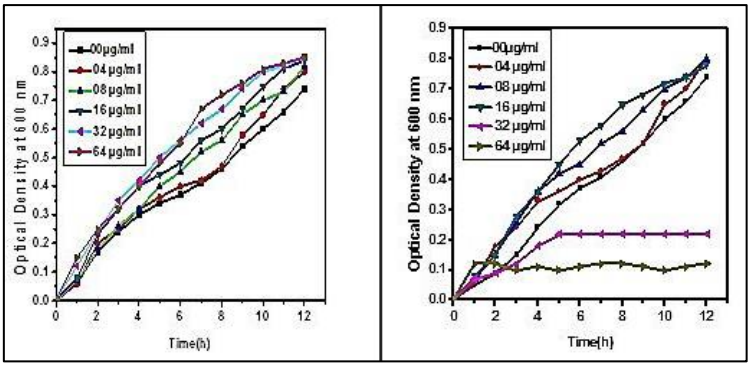


Figure 3: Growth curves of *E. coli* in LB medium with different concentrations of silver nanoparticles in the absence of irradiation and presence of irradiation: 0 µg/ml, 4 µg/ml, 8 µg/ml, 16 µg/ml, 32 µg/ml and 64 µg/ml, measured over a period of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 hrs

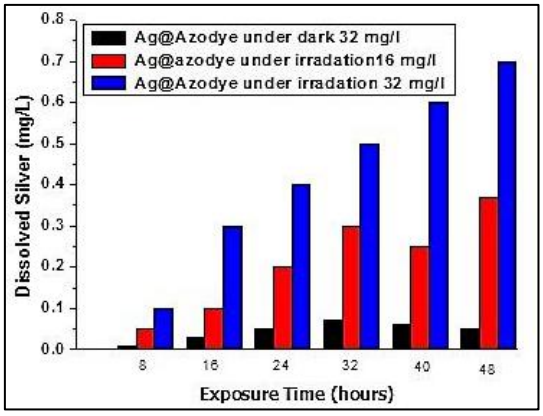


Figure 4: Atomic absorption spectroscopic studies of release of silver ions in water from Ag@azodye nanoparticles under dark and irradiation of UVA light for 16 and 32 mg/L nanoparticle

Ag@Azodye suspensions under dark exhibited very low dissolution with dissolved silver levels reaching a maximum value of 0.05 mg L^{-1} after 48 hours for 32 mg/L concentration of silver nanoparticles. In comparison, Ag^{+} concentrations in UVA irradiated suspensions increased to 0.7 mg L^{-1} after 48 hrs. It is also well known that plasmon resonance band of silver nanoparticle overlap with absorbance bands of azobenzene. The plasmon band of silver monolayer protected cluster is near 440 nm, which also corresponds to the n - π* transition of the N = N bond in azobenzene, this enables transfer of energy between the two species when azobenzene is brought close to the nanoparticle surface Figure 4. With increase in concentration the dissolution of silver was also increased, for 32 mg/L of Ag@Azodye the dissolution of silver was found to be 0.7 mg/L. Based on the observations from atomic absorption studies, the mechanisms by which azodye sensitized silver nanoparticles could show the antibacterial activity under UV light irradiation can be discussed. The silver ions produced on irradiation in bacterial plasma or cytoplasmic membrane may interact with a number of electron donor functional groups such as thiols, phosphates, hydroxyls, imidazoles and indoles contained in enzymes and DNA. As a result it inhibits cell division, damage the cell envelope and cellular contents of the bacteria, because of this bacterial growth is inhibited. The other factor which leads to bactericidal effect of these nanoparticles is generation of reactive oxygen species (ROS) forming free radicals with a powerful bactericidal action. Further studies are required to investigate of mechanism of action.

CONCLUSION

We have synthesised heterocyclic azodye functionalized nanoparticles, which exhibit enhanced activities against Gram-negative bacteria under photoinduced condition. The formation of functionalized silver nanoparticles was verified with UV-vis absorption spectra and it was found these nanoparticles to be photoresponsive, morphology of nanoparticles from TEM images was found to be spherical, the size of nanoparticle was found to be 9.5-16 nm, from XRD the nanoparticle was found to have fcc structure. *Invitro* bactericidal test showed that nanoparticles exhibited notable activity against *E. coli* under photoinduced condition, MIC of functionalized

silver nanoparticles under photo irradiation was found to be 32 µg/mL, the mechanism was proposed on the basis of atomic absorption studies.

ACKOWLEDGEMENT

The authors are thankful to the Chairman, Department of Industrial Chemistry, Kuvempu University, Shankaraghatta for providing the laboratory facilities.

REFERENCES

[1] F Carolin; L Melanie; R Helmut. *Polymers*. **2015**, 7, 717.
[2] SM Burkinshaw; M Paraskevas. *Dyes Pigments.* **2011**, 88(3), 396.
[3] Y Deng; Y Luo; P Wang; Y Lu; H Ming; Q Zhang. *Chinese Physics Lett*. **2007**, 24, 10.
[4] JW Christopher; P Prakash; D Tony; J James. *Chem Soc Perkin Trans*. **2002**, 1, 462.
[5] ZS Hoda; MM Rafat; HH Maher; EM Amira. *Molecules.* **2011**, 16, 6271.
[6] CT Keerthi Kumar; J Keshavayya; Rajesh; SK Peethambar. *Int J Pharm Pharm Sci*. **2013**, 5, 296.
[7] D Yonghong; L Youfa; Q Yong; Z Weijian; Q Xueqing. *ACS Sustainable Chem Eng*. **2015**, 3(6), 1111.
[8] A Paivi; JS David; KKJ Paprotny. *Phys Chem Chem Phys*. **2007**, 9, 651,
[9] M Lufsyi; S Edi; BSU Agung; AJ Kamsul. *Mod Phy*. **2015**, 6, 1071-1076.
[10] MR Lutfor; G Hegde; S Kumar; C Tschiersked; VG Chigrinov. *Mol Cryst Liq Cryst*. **2009**, 32, 176.
[11] C Meiwan; Y Zhiwen; W Hongmei; P Xin; X Xiaobao; W Chuanbin. *Int J Nanomedicine*. **2011**, 6, 2873.
[12] KJ Woo; CK Hye; WK Ki; S Sook; HK So; HP Yong. *Appl Environ Microbiol*. **2008**, 74(7), 2171-2178.
[13] WEI QingShan; JI Jian; FU JinHong; SHEN JiaCong. *Sci China Ser B Chem.* **2007**, 50, 418-424.
[14] M Veerapandian; K Yun. *Appl Microbiol Biotechnol*. **2011**, 90, 1655.
[15] NB Ashley; S Kathryn; AS Tova; L Jiangrui; OO Sherine. *Appl Environ Microbiol*. **2012**, 78, 2768.
[16] G Geeta; S Sristy; SC Baldev; RC Saumya; M Shanmugam; RC Anirban. *Microb Cell Fact*. **2016**, 15, 25.
[17] TS Anelia; Mavrova; W Diana; AT Jordan; AL Lubomir. *Eur J Med Chem*. **2014**, 86, 676.
[18] R Jurupula; N Nagabhushana; D Udayakumar. *Eur J Med Chem*. **2015**, 106, 75-84.